



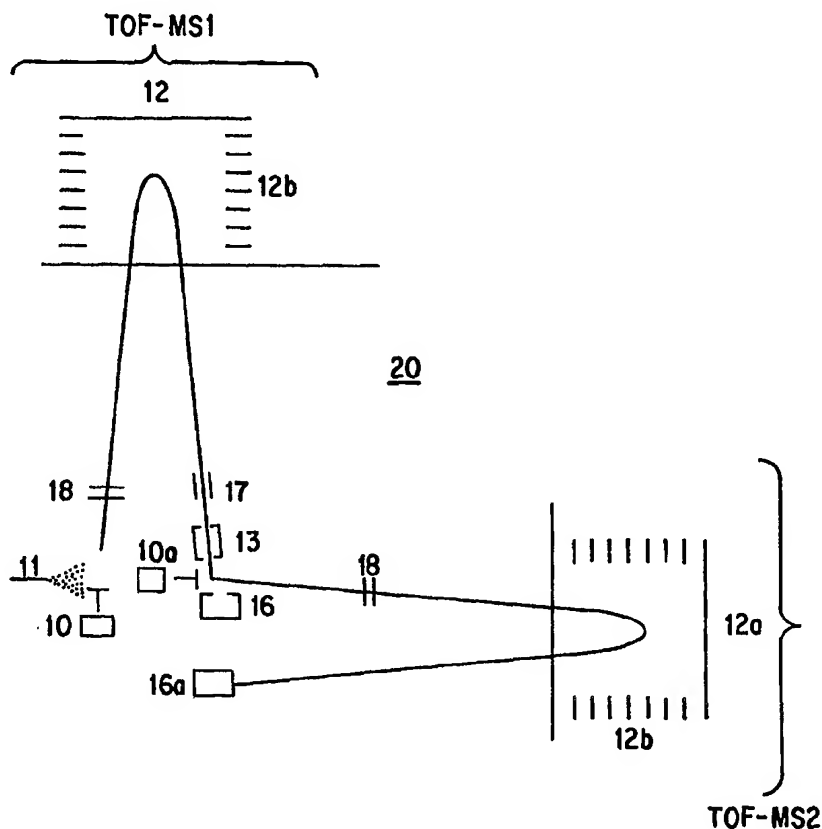
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US98/13545 (22) International Filing Date: 29 June 1998 (29.06.98) (30) Priority Data: 60/051,562 2 July 1997 (02.07.97) US (71) Applicant (for all designated States except US): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). (72) Inventor; and (75) Inventor/Applicant (for US only): HOP, Cornelis, E., C., A. [NL/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). (74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).			(81) Designated States: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, GW, HU, ID, IL, IS, JP, KG, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, SL, TJ, TM, TR, TT, UA, US, UZ, VN, YU, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>

(54) Title: NOVEL MASS SPECTROMETER

(57) Abstract

This invention is concerned with a tandem TOF/TOF mass spectrometer (20) which combines the advantages of Quadrupole Mass Spectrometer-TOF with high energy CID. The first part (TOF-MS 1) is an ion source (11) interfaced with an orthogonal TOF mass spectrometer-1 (12). The ions are orthogonally accelerated to a relatively high voltage, e.g. from about 1 keV to about 7 keV, preferably from about 2 to about 4 keV. In the second part (TOF-MS 2), the ions from the first part are then interfaced with an orthogonal TOF mass spectrometer-2 (12a) wherein the ions are orthogonally accelerated to a high voltage, e.g. from about 25 keV to about 50 keV, preferably from about 29 to about 35 keV.



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TITLE OF THE INVENTION
NOVEL MASS SPECTROMETER

BACKGROUND OF THE INVENTION

5 This invention relates to a tandem mass spectrometry system based on principles of analysis by time-of flight. The object is to indentify which molecules are present in a sample. The molecular weight of a molecule may be readily measured by ionizing the molecule and providing it with a fixed amount of kinetic energy and measuring
10 the time it takes for molecules to travel a fixed distance (time-of-flight). However a number of different molecules may have the same molecular weight and these can be distinguished from one another by dissociation and analysis of the molecular weight of the fragments that are produced. In recent years the need has arisen to analyze by tandem mass
15 spectrometry, with the highest sensitivities, complex biological and other molecules and complex mixtures of molecules.

 The various manufacturers of mass spectrometers have come a long way in accommodating scientists to get better quality data in a shorter period of time. Currently, the main area which drives
20 developments in mass spectrometry is biochemistry, in particular analysis of peptides, proteins and oligonucleotides. The technical requirements for mass spectrometers in order to meet the biochemical demand include high sensitivity, high scan speed, good mass accuracy, ease of use, and the ability to obtain structural information. MS/MS
25 (Tandem Mass Spectrometry) is the most common technique to obtain structural information by mass spectrometry. It is an improved form of mass spectrometry where a mass-selected ion beam (referred to as the parent ion beam) produced by a first mass analyzer is dissociated into a plurality of fragment ions. The fragment ions are then subjected to a
30 second stage of mass analysis, allowing molecular weight quantification of the various fragment ions. This information can be used to determine the structure of the parent ions.

 Although triple quadrupole (QQQ) mass spectrometers are the most common mass spectrometers in the biochemical community, they

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lack in scan speed and mass accuracy. Due to the relatively low scan speed it is difficult to interface a triple quadrupole mass spectrometer with capillary electrophoresis (CE) and capillary electrochromatography (CEC) equipment.

5 The recently introduced Micromass, Inc., (Manchester, U.K.) Quadrupole Time-Of-Flight (Q-TOF) mass spectrometer is an improvement over the triple quadrupole mass spectrometer. This instrument has an electrospray or atmospheric pressure chemical ionization (APCI) source interfaced to a quadrupole mass filter followed
10 by a hexapole gas cell and an orthogonal time-of-flight (TOF) mass spectrometer. In the MS mode the quadrupole mass filter operates in the RF (radio frequency) only mode and acts as a wide bandpass device. All ions are mass analyzed by the time-of-flight mass spectrometer. In the MS/MS mode the quadrupole is used to mass-select the ions of
15 interest. Next, these ions are collisionally dissociated in the gas cell and the fragment ions are mass-analyzed with the TOF section. This instrument has shown exceptional sensitivity in the MS and MS/MS mode especially in combination with nanoflow electrospray. The increase in sensitivity (about a factor of 30-50 compared to triple
20 quadrupole mass spectrometers) can be ascribed to the fact that all the ions in the spectrum are detected simultaneously, whereas with magnetic sector and quadrupole mass spectrometers the ions are detected consecutively according to their molecular weight-to-charge ratio (only one m/z ratio is detected at a time and all others are discarded). The
25 scan speed of the Q-TOF allows optimum use of the separation generated by CE equipment. Since a resolution of about 5,000 (full-width at half maximum definition) is standard, the mass measurements on the Q-TOF are usually within 10 ppm of the theoretical value. However, one of the disadvantages of a Quadrupole Time-Of-Flight
30 mass spectrometer, as well as triple quadrupole mass spectrometers, is that collision-induced dissociation (CID) is a low-energy event. Therefore, high energy processes such as side-chain fragmentation in peptides do not occur. This precludes differentiation between, for

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example leucine and isoleucine, and hampers identification of modified peptides.

The recently introduced Perseptive Biosystems Mariner (Framingham, MA.) Electrospray-TOF instrument displays excellent
5 sensitivity, resolution and mass accuracy. However, in this configuration MS/MS data cannot be obtained. Theoretically, some structural information can be obtained by post-source decay (PSD) of metastable or collisionally activated ions, with TOF mass spectrometers equipped with a reflector but this technique is cumbersome (a PSD mass
10 spectrum is obtained by stepwise reducing the reflector voltage and stitching all the partial spectra together) and the mass accuracy and resolution for the fragment ions is limited.

SUMMARY OF THE INVENTION

15 This invention is concerned with a tandem TOF/TOF mass spectrometer which combines the advantages of Quadrupole-TOF Mass Spectrometer with high energy excitation such as laser-induced photodissociation or CID. The first part (TOF-MS 1) is an ion source interfaced with an orthogonal TOF mass spectrometer-1. The ions are
20 orthogonally accelerated to a relatively high voltage, e.g. from about 1 keV to about 7 keV, preferably from about 2 to about 4 keV. In the second part (TOF-MS 2), the ions from the first part are then transferred to an orthogonal TOF mass spectrometer-2 wherein the ions are orthogonally accelerated to a high voltage, e.g. from about 25 keV
25 to about 50 keV, preferably from about 29 to about 35 keV. Because there is little energy spread in the orthogonal direction, the resolution obtained after passing through the reflector can reach more than 5,000 (full-width at half maximum definition) with an effective path-length of three or less meters. This invention is also concerned with an
30 orthogonal tandem TOF mass spectrometer that provides high energy CID which results in a broader range of fragmentation pathways than low energy CID, as well as high sensitivity, mass accuracy, and resolution. This invention is further concerned with an orthogonal

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tandem TOF mass spectrometer that is easy to manufacture and provides a small size footprint.

Thus, it is an object of the invention to provide an orthogonal tandem TOF/TOF mass spectrometer which is capable of combining the advantages of Q-TOF with high energy CID. Other aspects of the invention will become evident upon review of the disclosure as a whole.

DETAILED DESCRIPTION OF THE DRAWINGS

FIG. 1. Describes a tandem TOF/TOF mass spectrometer which contains two TOF-MS's, TOF-MS 1 and TOF-MS 2.

DETAILED DESCRIPTION OF THE INVENTION

Referring to FIG. 1, in one embodiment of this invention the novel orthogonal tandem TOF/TOF mass spectrometer (20) comprises an ion source (11), a first and second detector (16) and (16a), two time-of-flight analyzers (12) and (12a) each containing reflectors (12b), a first and second pusher (10) and (10a), an ion excitation device (13), and an electronic means for measuring the time-of-flight from the source to the detector, the point between the ion source (11) and the first detector (16) is referred to as TOF-MS 1 and the point between the second pusher (10a) and the second detector is referred to as TOF-MS 2, said first analyzer (12) is positioned between the ion source and the first detector, said second analyzer (12a) is positioned between the second pusher (10a) and the second detector (16a) in such a way that the ion path is orthogonal relative to the path of TOF-MS 1, said orthogonal ion path is controlled by pusher (10a) which is located after the excitation device (13) but before the second time of flight analyzer (12a), the first pusher (10) is positioned close to the ion source (11) and the excitation device is located after the first analyzer (12) but before the second pusher (10a). A high speed computer control system (e.g. pentium) controls the whole instrument.

In another embodiment of this invention the novel orthogonal tandem TOF/TOF mass spectrometer (20) optionally comprises a pulse deflection unit (17) positioned after the first time of flight analyzer (12)

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and before the excitation device (13) and is used when the sample is a mixture to mass select a particular ion prior to excitation.

In a preferred embodiment of this invention the mass spectrometer (20) comprises suitable transfer optics (18) to assist in
5 directing the path of the ions, said transfer optics (18) being located in close proximity to the ion source and second pusher. The design of the transfer optics will take account of the need to prevent excessive temporal pulse spreading, thereby maintaining high resolution in the TOF-MS's 1 and 2.

10 In yet another embodiment the invention can be said to comprise a method of orthogonal tandem TOF/TOF mass spectrometry comprising forming an ion flow along a path from an ion source (11), directing the path orthogonally relative to the ion source and accelerating it via a first
15 pusher device [high voltage electrode at about 1-7 keV, preferably 2-4 keV] (10) through a first time-of-flight analyzer (12) to an excitation device (13), the point between the ion source and the excitation device being referred to as TOF-MS 1, then accelerating the ion path (parent & fragment ions) orthogonally, relative to TOF-MS 1, to an energy (from
20 about 20 to about 50 keV, preferably 25-40 keV) much higher than the acceleration employed with the first pursher (10) using a second pusher (10a), sending the ion path through a second time-of-flight analyzer (12a) to a second detector (16a), and detecting ions at the detector including measurement of the time-of-flight of the ions.

In still another embodiment the invention can be said to comprise
25 a method of orthogonal tandem TOF/TOF mass spectrometry comprising forming an ion flow along a path from an ion source (11), directing the path orthogonally relative to the ion source and accelerating it via a first pusher device [high voltage electrode at about 1-7 keV, preferably 2-4 keV] (10) through a first time-of-flight
30 analyzer (12) to a pulse deflection unit (17) then to an excitation device (13), the point between the ion source and the excitation device being referred to as TOF-MS 1, then accelerating the ion path (parent & fragment ions) orthogonally, relative to TOF-MS 1, to an energy (from about 20 to about 50 keV, preferably 25-40 keV) much higher than the

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acceleration employed with the first pusher (10) using a second pusher (10a), sending the ion path through a second time-of-flight analyzer (12a) to a second detector (16a), and detecting ions at the detector including measurement of the time-of-flight of the ions.

5 In yet another embodiment the method invention can be said to comprise a method of orthogonal tandem TOF/TOF mass spectrometry wherein suitable transfer optic means are employed and are located in close proximity to the ion source and the second pusher.

10 The means of producing ions may be electron impact ionization, chemical ionization, field ionization, field desorption, atmosphere pressure ionization (such as electrospray, nebulizer-assisted electrospray, ionspray, atmospheric pressure chemical ionization and sonic spray), ion or atom bombardment (fast atom bombardment), laser desorption (including matrix assisted laser desorption) or resonance-
15 enhanced multiphoton ionization. The ions generated in the ion source (11) are pulsed into the tandem mass spectrometer by the first pusher so that ions are formed only within defined time intervals. Excitation of ions may be through collision with a gas (e.g. CID) or through laser-induced photodissociation. Either excitation process generates fragment
20 ions, which can be mass-analyzed in the MS/MS mode.

A primary mass spectrum may be obtained by transferring the ions from the ion source (11) to the first detector (16) without excitation and measuring flight-times along the sections of the path such as from the ion source (11) to the first detector (16) for the different
25 ions. Typically the mass spectrum is obtained by counting the number of ions striking the detector in each time interval. Here pusher (10a) is off.

Tandem mass spectra (MS/MS) may be obtained in a variety of ways. Here pusher (10a) is on and the first detector (16) can be on or
30 off, preferably off. A pulse deflection unit (17) positioned after the first time-of-flight analyzer (12) and before the excitation device (13) can be used to select a particular ion prior to excitation. Fragmentation is induced by excitation, the ions are then accelerated orthogonally to an energy of from about 20 to about 50 keV using pusher (10a) and the

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tandem mass spectrum for that selected ion is measured using TOF-MS (2). When the first detector (16) is on it is used to detect the neutrals in the MS/MS mode. The second detector is used to get the MS/MS spectrum of the mass selected parent ion.

5 TOF-TOF's may be fully computer controlled and mass assignment may be performed automatically by the computer based on prior mass calibration.

The instant TOF-TOF comprises two reflectors or reflecting electrostatic mirrors. An electrostatic mirror may be of a type described
10 by Manyrin et al (Sov. Phys. JETP 37, 45-48 (1973) providing a degree of energy compensation and little spatial defocussing or a type described by Hamilton et al (Rev, Sci Instrum., 61, 3104-3106 (1990) providing full energy compensation of an ion related to its mass-to-charge ratio even if ions of different masses have identical velocity.
15 Alternatively, the instant TOF-TOF does not contain reflectors or reflecting electrostatic mirrors.

TOF-TOF may be applied to either positive or negative ions and provides an infinite mass range. TOF-TOF also provides capabilities which cannot be achieved using magnetic sector instruments or using
20 quadrupoles and finds particular application in the analysis of large molecules, for example in biotechnology, biochemistry, and the like as well as in the analysis of mixtures. It will provide sensitivity greater than that achievable by other tandem mass spectrometry systems such as 4-sector or triple quadrupoles especially where a limited amount of
25 sample is available.

This instrument will be particularly useful for protein and peptide sequencing. Proteins can be enzymatically or chemically digested and the molecular weights of the generated peptides can be determined by the instant invention in the MS mode. The amino acid sequence of
30 peptides can be obtained by mass-selecting them, by the pulse deflection unit, followed by, for example, collision-induced fragmentation and mass analysis of the fragment ions. Since peptides fragment in a sequence specific manner, the sequence can be derived from the observed fragment ions.

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WHAT IS CLAIMED IS:

1. A novel orthogonal tandem TOF/TOF mass spectrometer comprising an ion source, a first and second detector, two time-of-flight analyzers, a first and second pusher, an ion excitation device, and an
5 electronic means for measuring the time-of-flight from the source to the first and second detectors, the point between the ion source and the first detector is referred to as TOF-MS 1, said first analyzer being positioned between the ion source and the first detector in such a way that the ion
10 path is orthogonal, said second analyzer being positioned between the second pusher and the second detector in such a way that the ion path is orthogonal relative to the path of TOF-MS 1, said orthogonal ion path being controlled by the second pusher which is located after the
excitation device but before the second time of flight analyzer, the first
15 pusher is positioned close to the ion source and the excitation device is located after the first analyzer but before the second pusher.

2. A mass spectrometer according to claim 1 which optionally comprises a pulse deflection unit positioned after the first time of flight analyzer and before the excitation device and wherein the two time-of-
20 flight analyzers each contain reflectors.

3. A mass spectrometer according to claim 2 which comprises transfer optics to assist in directing the path of the ions, said transfer optics located in close proximity to the ion source and the second
25 pusher.

4. A method of orthogonal tandem TOF/TOF mass spectrometry comprising forming an ion flow along a path from an ion source, directing the path orthogonally relative to the ion source and
30 accelerating it via a first pusher device [high voltage electrode at about 1-7 keV, preferably 2-4 keV] through a first time-of-flight analyzer to an excitation device, the point between the ion source and the excitation device being referred to as TOF-MS 1, then accelerating the ion path orthogonally relative to TOF-MS 1 to an energy (from about 20 to

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about 50 keV, preferably 25-40 keV) much higher than the acceleration employed with the first pursher using a second pusher, sending the ion path through a second time-of-flight analyzer to a second detector, and detecting ions at the detector including measurement of the time-of-flight of the ions.

5
10 5. A method according to claim 4 wherein transfer optics are employed to assist in directing the path of the ions, said transfer optics being located in close proximity to the ion source and the second Pusher.

15 6. A method according to claim 4 wherein a pulse deflection unit positioned after the first time of flight analyzer and before the excitation device is optionally employed and used in selecting a particular ion prior to excitation.

20 7. A method of orthogonal tandem TOF/TOF mass spectrometry comprising forming an ion flow along a path from an ion source, directing the path orthogonally relative to the ion source and accelerating it via a first pusher device [high voltage electrode at about 1-7 keV, preferably 2-4 keV] through a first time-of-flight analyzer to a pulse deflection unit then to an excitation device, the point between the ion source and the excitation device being referred to as TOF-MS 1, then accelerating the ion path orthogonally, relative to TOF-MS 1, to an energy (from about 20 to about 50 keV, preferably 25-40 keV) much higher than the acceleration employed with the first pursher using a second pusher, sending the ion path through a second time-of-flight analyzer to a second detector, and detecting ions at the detector including measurement of the time-of-flight of the ions.

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8. A method according to claim 7 wherein transfer optics are employed to assist in directing the path of the ions, said transfer optics being located located in close proximity to the ion source and the second Pusher.

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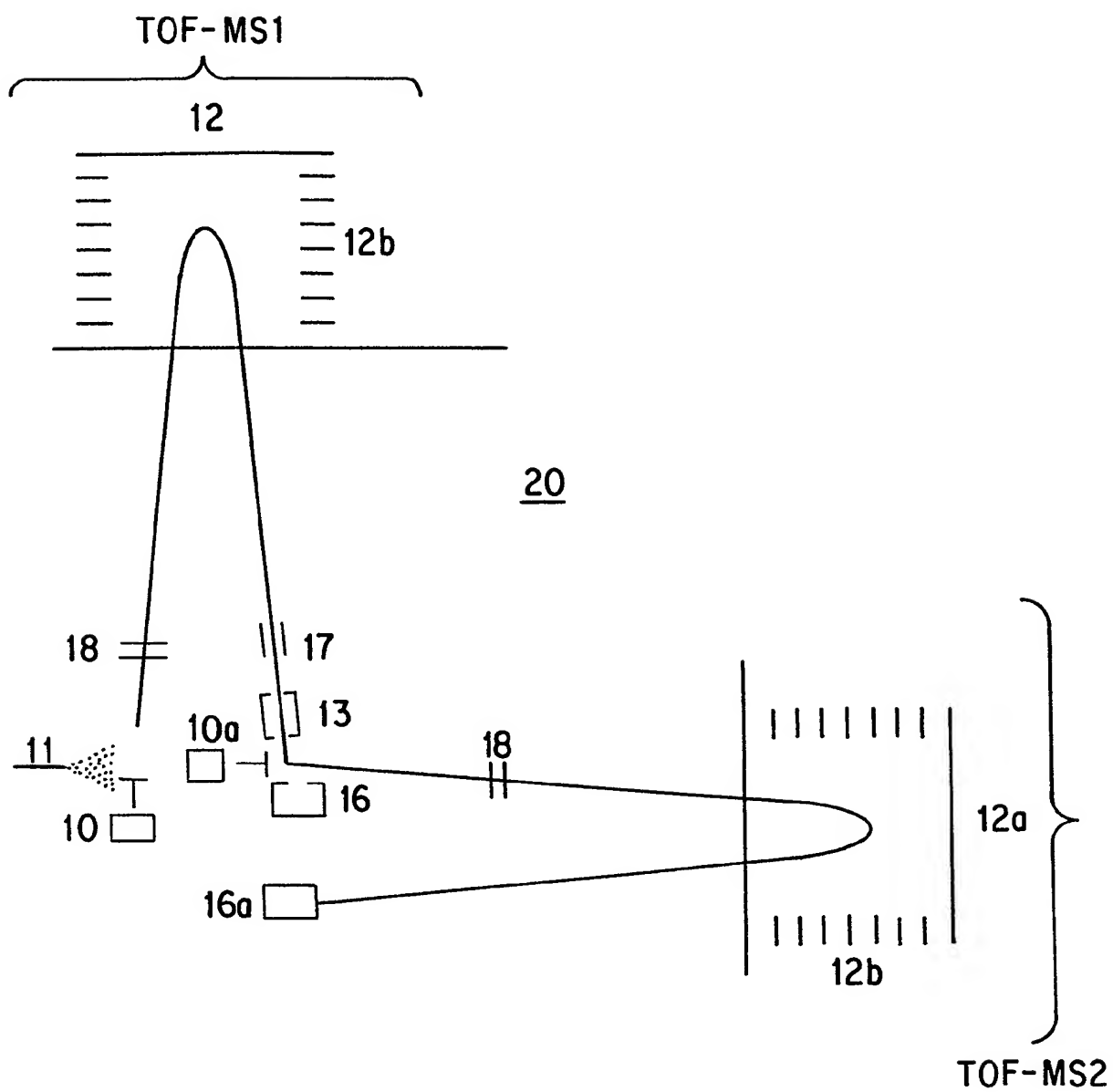


FIG.1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/13545**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : H01J 49/40

US CL : 250/287, 282

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 250/287, 282

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,202,563 A (COTTER et al) 13 April 1993 (13.04.93), whole document.	1-8
A	US 5,206,508 A (ALDERDICE et al) 27 April 1993 (27.04.93), whole document.	1-8
A	US 5,763,878 A (FRANZEN) 09 June 1998 (09.06.98) whole document.	1-8

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Further documents are listed in the continuation of Box C.

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See patent family annex.

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Facsimile No. (703) 305-3230

Authorized officer

JACK BERMAN

Telephone No. (703) 308-4849